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EFFECTS OF ANTIDEPRESSANT DRUGS ON NORADRENALINE ACCUMULATION AND CONTRACTILE RESPONSES IN THE RAT ANOCOCCYGEUS MUSCLE

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1 The effect of a series of antidepressant drugs on noradrenaline accumulation was studied in the isolated anococcygeus muscle of the rat.

2 The most potent inhibitors of noradrenaline accumulation were nortriptyline, desipramine and protriptyline. Opipramol, trimipramine and iprindole were active only in high concentrations.

3 Contractions of the anococcygeus muscle produced by noradrenaline were strongly potentiated by nortriptyline, desipramine and protriptyline. Other uptake inhibitors were less active in potentiating the noradrenaline response.

4 Nortriptyline, in concentrations that potentiated the action of noradrenaline, reduced or abolished the response to tyramine.

Introduction

The use of the isolated anococcygeus muscle of the rat in pharmacological research was first described by Gillespie (1972). This preparation appears to offer advantages over other sympathetically innervated isolated tissues and has been used successfully in studies of the actions of drugs acting on α-adrenoceptors and on catecholamine release and uptake processes (Gillespie, 1972; Gibson & Gillespie, 1973; Gibson & Pollock, 1973; Nash, Gillespie & Robertson, 1974; Gillespie & McGrath, 1974; 1975).

In the present paper, we describe the effects of a series of antidepressant drugs on the accumulation of radioactive noradrenaline in the anococcygeus muscle. We have also studied the effects of the same drugs on the responses of the muscle to exogenously applied noradrenaline and to the indirectly-acting sympathomimetic, tyramine.

Methods

Male rats (about 350 g) were used. The animals were killed and the anococcygeus muscles were dissected out as described by Gillespie (1972).

Effect of antidepressant drugs on noradrenaline accumulation

The accumulation of radioactivity in the tissues was measured as follows. The anococcygeus muscles were removed from rats. The muscle was mounted on a wire frame under a constant tension of approximately 0.2 to 0.5 g and placed in a tube containing Krebs solution at 37°C. The solution was gassed with 95% O_2 and 5% CO_2. Tritiated noradrenaline, suitably diluted with unlabelled compound, was added to a final volume of 5 ml. The mixture was incubated for the appropriate length of time at 37°C, then blotted and washed for 10 min in 5 ml of Krebs solution. The muscle was blotted dry, cut into 4 approximately equal pieces which were weighed. Each portion of muscle was placed in a test tube with 0.4 ml of 'protosolve' (NaOH 120 g in one litre methanol). When the tissue had dissolved, 10 ml of scintillation fluid and 0.25 ml of glacial acetic acid were added. The radioactivity was counted in a Phillips liquid scintillation counter. The radioactivity remaining in the medium after the incubation was determined by taking a 0.1 ml sample of medium and adding 0.9 ml of distilled water together with 10 ml of scintillation fluid and counting the radioactivity. The accumulation of radioactivity, expressed as nmol noradrenaline/ g tissue, and the tissue:medium ratios were then calculated.

Parallel experiments were performed in which the effect of various antidepressant drugs on the noradrenaline accumulation was studied. In these experiments the incubations were carried out in Krebs solution containing different concentrations of drugs. Inhibition of noradrenaline accumulation was expressed as a percentage of control values. Dose-response curves were plotted of % inhibition of noradrenaline accumulation against log M con-

centration of uptake inhibitor. The IC_{50} values (concentration causing 50% inhibition of noradrenaline accumulation) for each compound were calculated from regression lines.

Chromatography

Anococcygeus muscles were incubated for 20 min in 5 ml of Krebs solution containing 0.6 nmol of tritiated noradrenaline, then blotted and washed as previously described. After weighing, 4 muscles were pooled, cut into small pieces and ground in 0.35 ml of ice cold acetic acid (0.1 mol/l) with a glass grinder. The mixture was centrifuged and 30 µl of the supernatant was run on a thin layer chromatography plate using either *n*-butanol:water:acetic acid (12:5:3: by vol) or isopropanol:2M HCl (65:35 v/v) as the solvent system. The radioactivity of 1 cm strips of the chromatography plate was then determined. Unlabelled noradrenaline together with the major noradrenaline metabolites, was run concurrently in the same solvent systems. The position of the unlabelled material was located by the use of diazotised *p*-nitroaniline as described by Fleming & Clark (1970).

Drugs

(–)-[7-^{3}H]-noradrenaline hydrochloride (specific activity 10.3 Ci/mmol) was obtained from Amersham. Unlabelled (–)-noradrenaline bitartrate (Koch-Light), together with 200 ng (in 10 µl) of [^{3}H]-noradrenaline was made up to 1 ml in tartaric acid solution (1.5 mmol/l): 0.1 ml of this solution was made up to 5 ml with Krebs solution to give a final noradrenaline concentration of 61 pmol/ml. This final solution was kept under liquid nitrogen until required for use. Other concentrations were prepared accordingly by varying the amount of unlabelled noradrenaline added.

The other drugs used were tyramine hydrochloride, acetylcholine chloride (Koch-Light); carbachol (BDH); DL-3,4-dihydroxymandelic acid, DL-methoxy-4-hydroxymandelic acid DL-normetanephrine (Aldrich); amitriptyline hydrochloride, maprotiline hydrochloride, protriptyline hydrochloride, imipramine hydrochloride, desipramine hydrochloride, clomipramine hydrochloride, opipramol dihydrochloride (Ciba-Geigy); nortriptyline hydrochloride (Eli Lilly); doxepin hydrochloride (Pfizer) iprindole (John Wyeth).

The Krebs solution used had the following composition (g/l): $CaCl_2.6H_2O$ 0.55, KCl 0.35, KH_2PO_4 0.16, $MgSO_4.7H_2O$ 0.29, $NaHCO_3$ 2.1, NaCl 7.1 and glucose 1.0.

Dose-response curves on the anococcygeus muscle

Individual anococcygeus muscles were set up, with a resting tension of 0.2 to 0.5 g, in a 10 ml bath containing Krebs solution at 37°C. The preparation was gassed with 95% O_2 and 5% CO_2. Contractions of the muscle were recorded with an isometric transducer and a Servoscribe pen recorder.

Drugs were added to the preparation in a small volume (less than 0.3 ml) to give the final bath concentrations indicated. Dose-response curves were obtained as described by Gibson & Pollock (1973). Responses were expressed as a percentage of the maximum noradrenaline response which was between 3 and 7 g tension. A regression line was calculated for each dose-response curve over the range 20 to 80% of the maximum response. The pD_2 values (negative logarithm of M concentration of agonist producing 50% of maximum response) were obtained as described by Gibson & Pollock (1973). Additional dose-response curves were obtained to acetylcholine or carbachol.

When studying the effects of antidepressant drugs on the response, these drugs were present in the Krebs solution throughout the experiment; the tissue was allowed to equilibrate with the drug for 30 min before the experiment was started. pD_2 values for noradrenaline and tyramine in the presence of various antidepressant drugs were obtained in the usual way. Statistical evaluation was carried out using an unpaired *t* test.

The ability of drugs to potentiate the actions of noradrenaline are expressed as the dose-ratios (potentiation), which were obtained by taking the antilogarithm of the difference between the pD_2 values for noradrenaline obtained in the presence and absence of antidepressant drug. The ability of the same drugs to inhibit the response to tyramine is expressed, where possible, as the dose-ratio (inhibition), which was calculated in an analogous way.

Results

Time course of noradrenaline accumulation

The accumulation of radioactivity in the anococcygeus muscle following different times of incubation was measured at 3 different concentrations of noradrenaline (31, 61 and 122 pmol/ml). With these low concentrations uptake is likely to be mainly into adrenergic nerves, with no accumulation within smooth muscle (Nash *et al.*, 1974). The results obtained in these experiments are illustrated in Figure 1 where maximum tissue:medium ratios of 5.4 ± 0.8 (s.e. mean : $n=4$) were obtained with 61 pmol/ml. It can be seen that accumulation of radioactivity in the preparation increased with time and with increasing noradrenaline concentrations in the incubation fluid.

Identification of noradrenaline

In 4 separate experiments muscles that had been incubated with tritiated noradrenaline for 20 min were

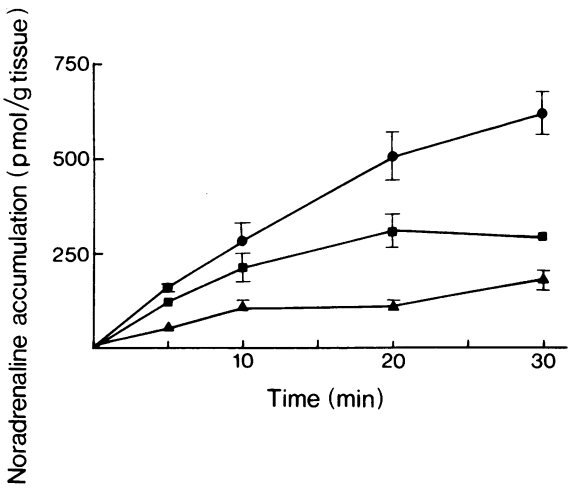

Figure 1 Time course of accumulation of radioactivity in rat anococcygeus muscle following incubation in tritiated noradrenaline at the following concentrations: (▲) 31 pmol/ml; (■) 61 pmol/ml; (●) 122 pmol/ml. Each point is the mean of 4 observations. Vertical lines show s.e. means.

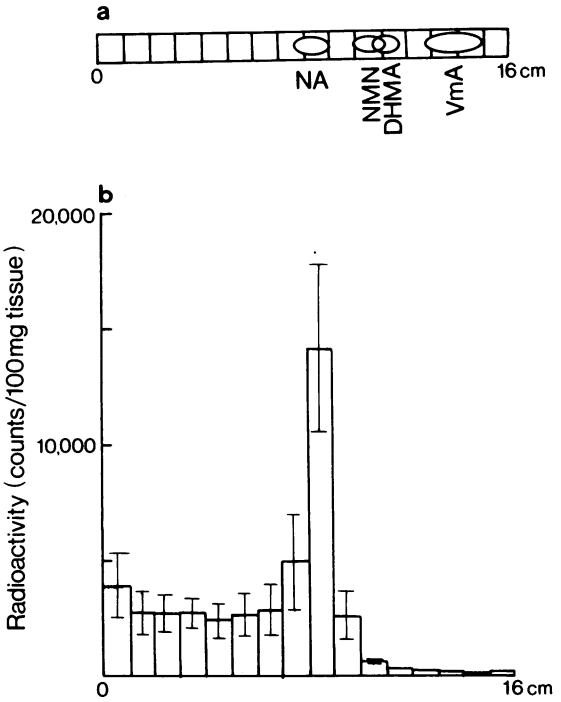

Figure 2 (a) Thin layer chromatogram of noradrenaline (NA) and some NA metabolites. Solvent system *n*-butanol:H_2O:acetic acid. NMN=normetanephrine; DHMA=3,4-dihydroxymandelic acid; VMA=3methoxy-4-hydroxymandelic acid. (b) Thin layer chromatogram of radioactivity extracted from anococcygeus muscle following incubation with tritiated noradrenaline. Solvent system as in (a). Note that the major peak of radioactivity corresponds to authentic noradrenaline. Values are the mean of 4 observations. Vertical lines show s.e. means. In (a) and (b) each plate was allowed to run 16 cm.

homogenized and an aliquot of the muscle homogenate was run on thin layer chromatograms in two separate solvent systems. In all cases radioactivity eluted from the plate corresponded to authentic noradrenaline, there being no evidence for the presence of any metabolites of noradrenaline (Figure 2).

Table 1 Inhibition of noradrenaline uptake by drugs. Potency is expressed as IC_{50} values and as the equipotent molar ratio (epmr) which is IC_{50} for drug/IC_{50} nortriptyline

Drug	*IC_{50} (M)*	*epmr*
Nortriptyline	1.0×10^{-8}	1
Desipramine	4.0×10^{-8}	4
Protriptyline	5.9×10^{-8}	6
Imipramine	2.9×10^{-7}	29
Doxepin	8.9×10^{-7}	89
Cocaine	9.2×10^{-7}	92
Clomipramine	1.2×10^{-6}	120
Maprotiline	1.3×10^{-6}	130
Amitriptyline	2.3×10^{-6}	230
Opipramol	1.9×10^{-5}	1900
Trimipramine	2.9×10^{-5}	2900
Iprindole	5.6×10^{-5}	5600

Values were calculated from regression lines and involved at least four separate determinations for each compound.

Inhibition of noradrenaline accumulation by drugs

The most potent inhibitors of noradrenaline accumulation were nortriptyline, desipramine and protriptyline. Each of these compounds caused 50% inhibition of uptake at concentrations of less than 10^{-7}M. The dose-response curves for inhibition of accumulation by nortriptyline, amitriptyline and iprindole are shown in Figure 3. The IC_{50} values, obtained from regression lines, for all compounds tested are shown in Table 1.

Effects of drugs on the response to noradrenaline

Noradrenaline caused powerful contractions of the anococcygeus muscle, as previously described

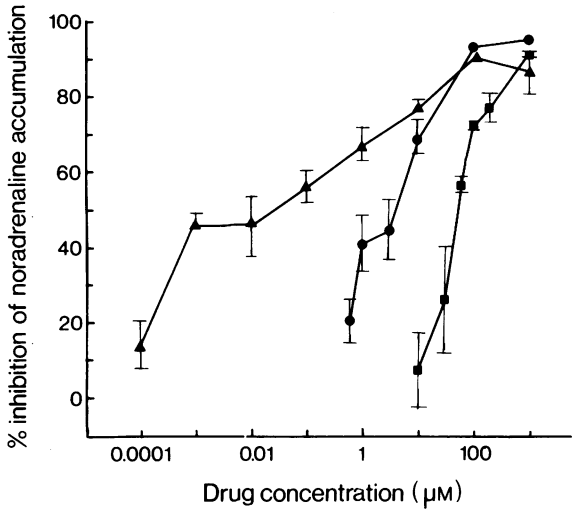

Figure 3 Dose-response curves for the inhibition by drugs of noradrenaline accumulation in rat anococcygeus muscle: (▲) inhibition by nortriptyline; (●) inhibition by amitriptyline; (■) inhibition by iprindole. Each point is the mean of 4 observations. Vertical lines show s.e. means. Noradrenaline accumulation was calculated as % of control accumulation occurring in the absence of drugs after incubation for 20 min in [^{3}H]-noradrenaline (61 pmol/ml).

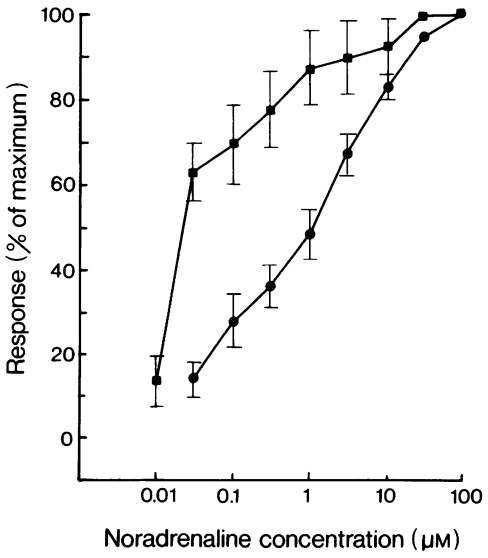

Figure 4 Effect of nortriptyline on the response to noradrenaline of the rat anococcygeus muscle. Responses are expressed as a percentage of maximum response: (●) contractions produced by noradrenaline alone; (■) effect of noradrenaline in the presence of 10^{-5} M nortriptyline. Each point is the mean of 4–6 observations. Vertical lines show s.e. means.

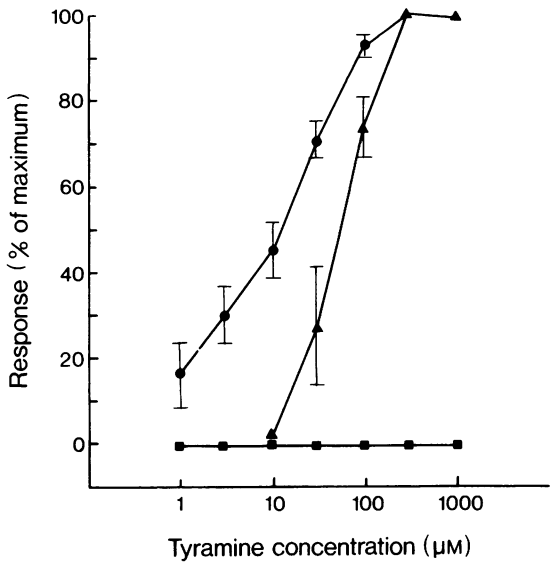

Figure 5 Effect of nortriptyline on the response to tyramine of the rat anococcygeus muscle. Responses are expressed as a percentage of the maximum response which was the same as that produced by 10^{-4} M noradrenaline. (●) Contractions produced by tyramine alone; (▲) effect of tyramine in the presence of 10^{-7} M nortriptyline. In the presence of 10^{-5} M nortriptyline (■) the tyramine response was completely abolished. Each point is the mean of 4–6 observations. Vertical lines show s.e. means.

(Gillespie, 1972). The pD_2 value for noradrenaline was 6.2 ± 0.2 (s.e. mean, $n = 10$), and maximum contractions were produced at 10^{-4} M. None of the antidepressant drugs caused contraction of the muscle in the concentrations tested.

The most active inhibitors of noradrenaline accumulation were also the most potent in terms of their ability to potentiate the actions of exogenously applied noradrenaline (Table 2). The degree of potentiation produced was usually greatest at a tricyclic antidepressant concentration of 10^{-6} or 10^{-7}M. The effect of nortriptyline on the noradrenaline response is illustrated in Figure 4. Maximum potentiation of the noradrenaline response was produced by 10^{-7} M nortriptyline.

Nortriptyline (10^{-7} M) reduced the response of the muscle to tyramine (Figure 5). At 10^{-5} M nortriptyline the response to tyramine (up to 10^{-3} M) was completely abolished, whereas the response to noradrenaline was still potentiated, although to a lesser extent than at 10^{-7} M. None of the drugs tested caused potentiation of the tyramine response. Clomipramine and opipramol had no potentiating actions but at a concentration of 10^{-5} M each of these

compounds reduced the response produced by noradrenaline.

The effects of the other compounds tested on the responses of the muscle to noradrenaline and tyramine are summarized in Table 2.

Effects of drugs on the response to acetylcholine and carbachol

Acetylcholine causes contraction of the anococcygeus muscle (Gillespie, 1972). In the present study the acetylcholine dose-response curves were not significantly shifted by any of the compounds listed in Table 2 (each at 10^{-6} M).

At a higher concentration (10^{-5} M) nortriptyline, protriptyline and doxepin antagonized the action of acetylcholine. Iprindole (10^{-5} M) potentiated responses to acetylcholine or carbachol.

Discussion

There are two main processes by which noradrenaline can be taken up by tissues. The neuronal system ($Uptake_1$) has a high affinity for noradrenaline. In addition, there is the lower affinity extraneuronal system, designated $Uptake_2$. (See reviews by Iversen, 1967; 1971a & b.)

In a recent study Nash *et al.* (1974) provided strong evidence that at noradrenaline concentrations below 2 μg/ml the rat anococcygeus muscle accumulates noradrenaline mainly into adrenergic nerves, with little contribution by $Uptake_2$. In the present study we have used low concentrations of noradrenaline in order to study mainly the neuronal uptake system. In our chromatographic experiments we have shown that at these low noradrenaline concentrations, all of the radioactivity retained in the muscle behaves as

Table 2 Potency of drugs in terms of their ability to potentiate the response to noradrenaline and inhibit the response to tyramine on the rat anococcygeus muscle.

Drug	*Concn (M)*	*Noradrenaline response Dose-ratio (potentiation)*	*Tyramine response Dose-ratio (inhibition)*
Nortriptyline	10^{-5}	25***	Cl
	10^{-6}	26**	5*
	10^{-7}	69**	7**
Protriptyline	10^{-5}	0	Cl
	10^{-6}	23**	12**
	10^{-7}	13**	0
Desipramine	10^{-5}	0	Cl
	10^{-6}	9**	6*
	10^{-7}	20***	0
Imipramine	10^{-5}	Redn	29***
	10^{-6}	14***	0
	10^{-7}	0	0
Doxepin	10^{-5}	0	Cl
	10^{-6}	13**	3*
	10^{-7}	0	0
Maprotiline	10^{-5}	5**	Cl
	10^{-6}	0	0
Amitriptyline	10^{-5}	0	Cl
	10^{-6}	7**	0
Trimipramine	10^{-5}	0	Cl
	10^{-6}	0	6**
Iprindole	10^{-5}	0	5
	10^{-6}	5**	0

Noradrenaline potentiation is expressed as dose-ratio (potentiation) and tyramine inhibition as dose-ratio (inhibition) (seeMethods section). Values quoted are the mean of at least 4 determinations.
Levels of significance (unpaired *t* test) when compared with control responses for noradrenaline or tyramine: *$P<0.05$; **$P<0.01$; ***$P<0.001$.
0=no significant difference from controls; Redn=noradrenaline response was reduced; Cl=complete or almost complete inhibition of tyramine response (thus not possible to calculate dose-ratios).

authentic noradrenaline, with no detectable presence of noradrenaline metabolites.

Many of the tricyclic antidepressant drugs are potent inhibitors of the neuronal uptake of noradrenaline (reviews by Biel & Bopp, 1974; Iversen, 1971a). Desipramine has been shown to be potent as a noradrenaline uptake inhibitor in rabbit aortic strips (Maxwell, Chaplin, Eckhardt, Soares, & Hite, 1970), rat brain slices (Salama, Insalaco & Maxwell, 1971), rat heart (Iversen, 1967) and in synaptosomes from rat brain (Horn, Coyle & Snyder, 1971; Ross & Renyi, 1975). Nortriptyline is similarly a potent noradrenaline uptake inhibitor, being only about 3 or 4 times less active than desipramine on rat brain synaptosomes and on rat heart (Iversen, 1967; Ross & Renyi, 1975), although slightly lower values for the potency of this compound have been reported by Horn *et al.* (1971) in their experiments on rat brain synaptosomes and by Maxwell *et al.* (1970).

In the results reported in the present paper nortriptyline and desipramine were powerful inhibitors of noradrenaline accumulation, nortriptyline being slightly more active than desipramine. The least active compound in our studies was iprindole, which was more than 5000 times less active than nortriptyline. This is in agreement with the work of others, who have shown that iprindole is weakly active as a noradrenaline uptake inhibitor (Gluckman & Baum, 1969; Lemberger, Sernatinger & Kuntzman, 1970; Lahti & Maickel, 1971; Ross, Renyi & Ogren, 1971). In spite of its low potency as an inhibitor of biogenic amine uptake, iprindole is a clinically effective antidepressant agent (for references see Biel & Bopp, 1974).

The most potent inhibitors of noradrenaline accumulation caused a large potentiation of the noradrenaline response in this tissue. That this potentiation is specific for the noradrenaline system is indicated by the fact that, with the exception of iprindole, none of the compounds tested caused potentiation of the response to acetylcholine or carbachol. Thus it is likely that, whereas iprindole may have a different mechanism of action, the other drugs potentiate the noradrenaline response by blocking noradrenaline uptake. A more direct way to study this would be to investigate the effects of the antidepressant drugs on an α-agonist which is not a substrate for uptake (Gibson & Pollock, 1973). However, this approach presents difficulties since drugs like oxymetazoline and methoxamine, which are not taken up presynaptically in this tissue, are nevertheless much more susceptible to α-blocking activity than is noradrenaline (Doggrell & Woodruff, unpublished observations).

The use of noradrenaline uptake inhibitors provides a means of distinguishing between directly-acting and indirectly-acting sympathomimetic amines. Thus in many tissues cocaine potentiates the actions of noradrenaline and some other directly-acting agonists but antagonizes the action of the indirectly-acting tyramine (see reviews by Iversen, 1971a; Trendelenburg, 1972).

In the anococcygeus muscle preparation cocaine potentiates the response to noradrenaline but the usefulness of this compound is limited by the fact that it releases noradrenaline and itself causes the muscle to contract (Gibson & Pollock, 1973; Gillespie & McGrath, 1974).

The use of tricyclic antidepressant drugs as uptake inhibitors is hampered in tissues containing α-adrenoceptors (Iversen, 1971a). Barnett, Symchowicz & Taber (1968) and Barnett, Staub & Symchowicz (1969), using the rat isolated vas deferens, have suggested that the antagonism of the tyramine response produced by imipramine and desipramine is due to the α-blocking activity of the drugs rather than to their ability to inhibit uptake. Hughes, Kneen & Main (1974), using the vas deferens of mouse and rat, have shown the effect of desipramine on the response of the tissues to noradrenaline is a balance between potentiation due to uptake block and antagonism due to receptor block. Such a balance is similarly indicated in our experiments. For example, desipramine, imipramine, doxepin and amitriptyline each caused potentiation of the noradrenaline response at 10^{-6} M but no potentiation at 10^{-5} M, presumably because at the high concentration the α-blocking activity of the compounds becomes significant. Nortriptyline has less α-blocking activity than desipramine and many other tricyclic antidepressants. This is shown in the experiments of Sturman (1971) who measured the potentiation produced by a series of tricyclic compounds on the response of the cat nictitating membrane to noradrenaline; nortriptyline caused a greater degree of potentiation than desipramine, imipramine or amitriptyline. The relatively weak α-blocking activity of nortriptyline is apparent in our experiments where in the presence of 10^{-5} M nortriptyline the noradrenaline response is still greatly potentiated.

There is evidence that the actions of tyramine on the anococcygeus muscle are mediated indirectly by the release of noradrenaline, since the responses are abolished by pretreatment with 6-hydroxydopamine (Gillespie & McGrath, 1975). With many of the uptake inhibitors used in the present study, the inhibition of the tyramine response is probably due to a combination of uptake inhibition and α-blocking activity. However, it is clear that using nortriptyline it is possible to distinguish between the action of noradrenaline and that of the indirectly-acting tyramine on this preparation.

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